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Pathogenesis of Fever

IVAN L. BENNETT, JR.

Pathologist-in-Chief, The Johns Hopkins Hospital

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*Studies on the Pathogenesis of Immunoallergic Arthritis Induced By Avian Nephrotoxic Serum in Rabbits**

CONSTANTIN V. TEODORU

AND

PAUL BRANCATO AND BRUNO W. VOLK

Isaac Albert Research Institute of the Jewish Chronic Disease Hospital
Brooklyn, New York

It has previously been shown that repeated injections of small amounts of Duck Anti-Rabbit Kidney Serum (DARKS) into the joints of rabbits produce a progressive, self-perpetuating arthritis which developed after a latent period averaging ten weeks[1-3]. When Normal Duck Serum (NDS) was given under similar circumstances, arthritis failed to develop. Moreover, when administered intradermally or subcutaneously, DARKS spared both the kidneys and joints, but elicited skin lesions which, again, could not be produced with NDS.[1, 4] This diversity in the location of lesions following different routes of administration suggested that DARKS may react with an antigenic element present not only in kidneys but also in other organs and tissues.

It was, therefore, attempted to investigate the tissue elements with which DARKS might be reacting, to study the secondary response of the host rabbit and to establish the role of the nephrotoxic principle and that of the host response in the pathogenesis of the joint disease. For this purpose the local reactions were studied clinically

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and morphologically at varying intervals following the intra-articular administration of 0.5 ml. of serum given twice weekly over a period of six weeks. The immune response of the host rabbit was evaluated by means of precipitin reactions occurring between the host-rabbit serum and either whole duck serum or isolated duck gamma globulins. The affinity of DARKS for various tissues was studied by means of fluorescein-labeled globulins isolated from DARKS, from NDS and from the serum of rabbits preimmunized against duck globulins[5]. Alterations in the pattern of the host response to duck serum were induced by means of conditioning factors such as pre-immunization, high NaCl and high KCl intake.[6]

About ten weeks following the first, or four weeks following the last intra-articular injection of DARKS, the joint became swollen, the bone prominences appeared padded by edematous tissue, and pain and functional impairment were obvious. Radiologic examination showed, at first, bulging of the articular capsule and, later, various degrees of cartilage and bone involvement, as indicated by narrowing of the joint space, hyperostosis, osteosclerosis or, in some animals, osteoporosis.

Morphologic studies of the joints showed fibromyositis of the hamstring muscles and synovial reaction and, at later stages, involvement of cartilage and bone. The myositis was characterized by infiltration of the muscle with lymphocytes, plasma cells and mononuclear cells. The synovium showed thickening and villous-like hypertrophy. Furthermore, the synovial tissue exhibited increased vascularity, infiltration with round cells and, at times, with polymorphonuclear leukocytes. With progression of time the cartilage became ulcerated, beginning at the site of attachment with the synovium. Eventually, the ulceration spread over the entire articular surface and in some instances formed a pad-like thickening. In several animals necrosis of the cartilage led to exposure of the underlying bone.

The immune response of the host rabbit followed a definite pattern. Anti-duck serum precipitins appeared one week following the intra-articular administration of either DARKS or NDS. The precipitin reactions were identical, regardless of whether DARKS, NDS, or isolated DARKS globulins were used for precipitation reaction. The circulating precipitins always preceded the development of arthritis. The precipitin level rose progressively in the rabbit serum and, after reaching a peak, decreased slowly and eventually disappeared. The peak and duration of the precipitin level increased in proportion to the amount and number of intra-articular injections. With equal treatment the precipitin titer dropped faster in those animals which developed arthritis than in those showing no clinical symptoms. This suggested fixation of the antibodies at the site of the affected joint.

Fluorescent DARKS-globulins, incubated with fresh sections of various rabbit organs, stained selectively the perivascular and interstitial connective tissue. In striated muscle, the peri- and endomysium stained brightly. The intima of the arteries also became fluorescent but only in rabbits pre-immunized against duck serum, and only subsequent to the appearance of circulating anti-duck serum precipitins. Fluorescent NDS-globulins failed to stain any tissue except for the intima of the arteries in pre-immunized rabbits, hence indicating the presence of host-anti-duck antibodies at that level. Fluorescent antibodies against duck globulin incubated with sections of joint capsule three months after intra-articular treatment with DARKS clearly indicated the presence of DARKS-globulins affixed to the interstitial connective tissue. Under similar circumstances, duck-globulins could not be demonstrated in joint sections of animals given NDS.

In preconditioned animals, the intra-articular injection of DARKS was followed by an accelerated arthritis. The rabbits pre-immunized against duck serum six to nine months previously developed arthritis within one week after the intra-articular injection of either DARKS or NDS. Circulating anti-duck precipitins appeared in such animals within 48 hours, although no precipitins were demonstrable at the time of the injection. Animals preconditioned by a high intake of NaCl given as an 0.9 percent solution instead of drinking water. developed accelerated arthritis within two weeks after intra-articular administration of

DARKS. Animals drinking isotonic KCl solution developed arthritis within three weeks following injection of DARKS. All conditioned rabbits showed circulating anti-duck serum precipitins preceding the development of arthritis. The precipitins appeared earlier and at higher levels in conditioned than in control animals, hence suggesting a direct relationship between accelerated antibody response and accelerated arthritis. The NaCl-conditioned animals showed the most severe arthritis. Gross examination of the joints revealed severe edema, formation of pannus and necrosis of cartilage which at times caused severe distortion of the affected joint. Histologically, severe synovitis, necrosis of articular cartilage, as well as villous hypertrophy of the synovium, were the principal pathologic changes. The villi became edematous and showed infiltration with lymphocytes and plasma cells and occasionally with polymorphonuclear leukocytes. Frequently, the synovial membranes displayed fibrinoid necrosis and were covered with an exudate consisting of lymphocytes and plasma cells and, in some instances, polymorphonuclear leukocytes. Eventually, this exudate was seen to cover a large area of the articular cartilage. In several instances, the cartilage became eroded and ulcerated with exposure of the subchondral bone tissue. Occasional animals showed miliary granulomas in the hamstring muscles adjacent to the joint cavity; these granulomas consisted mainly of lymphocytes and plasma cells. None of the animals displayed changes in other organs, including the blood vessels of kidneys.

The salt-conditioned animals developed arthritis also when normal duck, calf or horse sera were injected bi-weekly for at least three weeks. However, when corresponding amounts of concentrated globulins extracted from either DARKS or NDS were injected intra-articularly in a single 2 ml. dose, only animals receiving DARKS-globulins developed arthritis. This indicates that the arthritis induced by NDS in salt-conditioned animals depended upon the repeated administration, rather than on the quality of the injected serum, hence, suggesting an Arthus-type reaction. Since arthritic changes failed to occur, or were not manifest clinically in nonconditioned animals, it was hypothesized that the accelerated antibody response observed in the salt-conditioned rabbits was responsible for the enhancement of the Arthus-like reaction.

In conclusion, these experiments demonstrate that DARKS-globulins have a specific affinity for rabbit connective tissue, including synovium, perimysium and vascular adventitia, to which they remain affixed for extended periods of time. The linkage of these specific duck-globulins to interstitial connective tissue was not accompanied by local tissue damage until the host rabbit developed circulating anti-duck precipitins. At that time, duck-globulins could be demonstrated by the fluorescent antibody technique also in the intima of small and medium sized arteries, presumably linked to the specific antibodies developed by the host. Conditioning factors, such as pre-immunization or high salt intake, which accelerated and enhanced antibody formation, commensurately hastened the development of arthritis. The tissue reactions were of immunoallergic type, with predominance of round-cell infiltration and villous hypertrophy of the synovium. The site of lesions shown by routine histologic preparations closely corresponded to the site of duck-globulin fixation as demonstrated by fluorescent antibody studies. The experiments on conditioned animals also demonstrated that certain Arthus-like immunoallergic reactions, which usually do not occur or are too mild to be detected in normal animals, could be enhanced to the point of producing extensive pathologic lesions by means of conditioning factors such as a high-salt diet.

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